PART II

Conditions and Reactions in Muscle

I. Introduction

Up to this point we have discussed only the behavior of the contractile substance, extracted by different chemical manipulations. We came closest to the fibril by imitating its elongated shape and preparing threads from our actomyosin. In now discussing the fibril we must bear in mind that conditions in the muscle fibril differ widely from conditions in threads. Extracted actomyosin is no longer the same substance that existed in the muscle. Nor can the conditions of intermicellar spaces of muscle be imitated *in vitro*. The main difference is that this space, in muscle, is very limited and relatively small compared to the mass of actomyosin. So changes in adsorption of dissolved substances to actomyosin will change concentrations in the intermicellar space considerably. In the thread there is a little solid matter (2%) surrounded by a relatively large quantity of water (98%) so that the adsorption of substances to the solid will not alter appreciably the concentration of the solution.

Muscle contains around 10% actomyosin, located in the fibrils which occupy about half of the total volume.* The fibrils thus contain approximately 20% actomyosin. They probably contain 10% dissolved protein (myogen) which leaves 70% of their volume for intermicellar water. About half of the intermicellar water of the muscle fibre is located in the fibril.

Muscle contains 0.005 M myosin, calculated on the basis of the UW. It contains 0.005 M ATP bound to myosin. If there is no other protein to compete with myosin for the adsorption of ATP, then every UW of myosin holds one ATP molecule adsorbed. This is the quantity of ATP which, according to our previous experience, gives myosin optimal reactivity.

Muscle contains 0.002 M actin (calculated on the basis of the UW). This actin, if prepared according to Straub's method, contains one Ca and two Mg per UW. Since the method gives

* According to Dr. Buchthal's estimate, quoted by A. Krogh in his Croonian lecture, 60% of the volume of the muscle fiber is taken by fibrils. If the muscle contains 10% of blood vessels, fasciae, etc., then half of its total volume can be calculated as being occupied by the fibrils.

no opportunity for these ions to be bound in the course of preparations and since the actin, in this method, is treated with high concentrations of KCl, it is most probable that actin binds these metals in muscle too; that these cannot be expelled by alkali metals, and are bound in a specific way. It was indicated by Straub's experiments that six positive charges are needed to neutralize the basic charge of actin at pH 7. Actin is thus probably held in isoelectric condition *in vivo* by its bound bivalent ions.

According to Hill and Kupalow, frog muscle contains 0.007 M Ca and 0.014 M Mg. These values are somewhat higher than those given by M. Dubuisson (1942) for rabbits (0.005 M Ca and 0.01 M Mg). Taking Hill and Kupalow's values as a basis for calculation, the total concentration of Ca and Mg is 0.021 M of which 0.006 M is bound by actin, leaving 0.015 M free. If the 0.005 M myosin binds two M of this (four equivalents) per UW, 0.005 M thus becomes free, which will be largely Mg. Calculated for the 77% water contained in muscle, the concentration will be approximately 0.006 M. The curves of Banga show that myosin, with four equivalents of Mg or Ca bound, is actually in equilibrium with such a solution. The total concentration of free Ca^+ Mg cannot exceed 0.006 M, and in the muscle we can expect to find the basic negative charge of both actin and myosin balanced by the adsorbed bivalent ions, their primary adsorption being satisfied by Ca and Mg, the concentration and quantity of which is just sufficient to fulfill this role. On no account is there enough Ca or Mg to expel K from its secondary adsorption.

At the concentration of $K^+ + Na^+$ present in muscle, myosin can be expected to adsorb three alkali-metal ions in its secondary circle, which enables the myosin to adsorb ATP. I do not want to stress the actual numerical values which may be subject to modification. What is more important, at the moment, is the method of approach in calculating actual ionic concentrations which depend greatly on the adsorption by actin and myosin.

As mentioned before, there are considerable differences between the actomyosin found in threads and that found in

muscle. First, its distribution is different. The fibril is a highly organized structure with a high steric specificity. In the thread the myosin and actin particles are intermingled and distributed at random and, consequently, if the actomyosin dissociated, both actin and myosin dissolve. This behavior differs from that in muscle. If muscle is treated with strong salt-solution, the myosin dissolves and the actin remains. This suggests that in muscle, actin and myosin each forms a continuous system and it is not the myosin micels which unite with the actin micels to form actomyosin, but it is the myosin system which unites with the actin system. If the elementary filaments, which seem to build up the fibril (Hall, Jakus, and Schmitt) consist of actomyosin, they must be double threads, composed of a myosin and an actin thread.

As the experiments of Banga and Hermann have shown, the elementary processes, like adsorption by myosin, are not very different in extracted myosin from myosin in muscle. This, however, does not hold for more complex colloidal phenomena like dissociation. As shown, actomyosin — *in vitro* — dissociates in the presence of 0.1% ATP if the KCl concentration exceeds 0.16 M. Muscle fibres — washed or unwashed — contract up to 0.45 M KCl. They cease to contract and dissociate only if the KCl concentration reaches 0.5 M. A much higher charge and hydration is needed to separate actin from myosin in muscle, which shows that the forces holding these proteins together are considerably greater in muscle than in the extracted proteins and that these links, once disrupted, cannot be restored.

On extraction the myosin system disintegrates into small and uniform particles. This extracted myosin, in high concentrations, forms a soft, plastic mass which readily dissolves in water. This shows that *in vitro* the forces binding myosin to myosin are exceedingly low and do not explain the great resistance of muscle to tearing. It is highly probable that in muscle not only is actin linked to myosin by forces greater than those in the extracted condition, but also the myosin particles themselves form a continuous system, connected by links which once broken cannot be restored.

II. Rest

In discussing this basic condition of muscle, our first question may be whether actomyosin is dissociated or not: whether resting muscle contains actin and myosin side by side, or contains actomyosin. In my earlier papers I held the view that actomyosin must be dissociated. Resting muscle is soft, whereas a 20% actomyosin gel must be rigid. Moreover I was impressed by Hürthle's observation of a nematode moving with ease through the muscle fibre. No nematode could walk at ease through a 20% actomyosin gel. I did not then take into consideration two facts: one was the division of this actomyosin into very thin threads, which might make it easier for the nematode to get through; the other was the action of ATP. Resting muscle contains high concentrations of ATP, which must have a profound influence on the physical state of actomyosin turning the rigid gel into a soft, plastic mass. So both arguments in favor of dissociation have lost validity. The very strong resistance of muscle to tearing also indicates undissociated actomyosin.

The third argument which made me believe that actomyosin in resting muscle is dissociated was Varga's observation which definitely showed that actomyosin at body temperature must be contracted if ATP is present. The fact that muscle contains ATP in rather high concentration seemed to exclude the presence of undissociated actomyosin.

All would have been well had not the muscle fibre, washed or unwashed, readily contracted under the influence of ATP added from without. If actomyosin, *in vitro,* contracts under the influence of ATP, it is natural that it should do the same in the muscle. But if the fibre contracts under the influence of ATP, then, evidently, it cannot be dissociated, or else we should have to suppose that the first action of ATP is to cause association, which is contrary to experience. Therefore in a later paper I held the view that actomyosin, in resting muscle, cannot be

dissociated. I will not take sides here but will simply describe what can be seen.

If ATP, in resting muscle, does not cause contraction of actomyosin, then evidently either the ATP or the actomyosin must be present in an inactive condition. It was natural that the first thought should have been that ATP is present in an inactive modification; its formula offers rich possibilities for such isomerism. Efforts, however, to isolate such an inactive ATP failed [Rózsa, 44].

Erdös [70] and Rózsa [44] found that the ATP, present in resting muscle, is not completely inactive, but that its activity depends on the salt concentration. In isotonic 0.16 M KCl, NaCl, or Ringer's solution, the ATP was inactive and did not cause contraction of freshly isolated muscle fibres, but did so if the fluid was slightly diluted. Dilution by no more than 10% sufficed to induce violent contraction. The behavior of the myosin, with its own ATP, was thus analogous to the behavior of actomyosin threads in ATP. This analogy is made clear by Fig. 40. The upper half of this figure is identical with Fig. 20, drawn on a logarithmic scale. In the lower half the behavior of freshly isolated muscle fibres is shown. The zone where all fibres contracted is black. This zone is bounded on the right by a very narrow zone, and on the left by a wider zone where some of the fibres contracted, evidently owing to imperfections of the technique and the unavoidable excitation: In the figure this contraction is marked with lines. Muscle is thus balanced between contraction and relaxation, just on the side of the latter, and the isotonic concentration is the critical concentration which just maintains relaxation.

Results with frog muscle were analogous to those obtained with rabbits, the difference being that here the critical salt concentration lay 30% lower.

If ATP is added to a fresh muscle fibre suspended in isotonic solution, contraction occurs, and as the curve in Fig. 40

With rabbits, advantage was taken of the fact that the muscle is unexcitable at 0°. Frog muscles are very excitable and readily contract at 0°, so the isolation of single fibres was omitted and the muscle (gastrocnemius) was perfused *in situ* from the artery. If the Ringer solution was diluted by 15%, single electric shocks provoked, instead of a twitch, a short contraction.

shows, contraction can be obtained up to 0.45 M KCl. This is not unexpected because washed muscle fibres also contract up to this limit. This limit is characteristic for actomyosin in muscle. The problem is rather why do the two ATP's, one present in muscle and one produced from without, behave differently, and why does the former cause contraction only below the isotonic concentration.

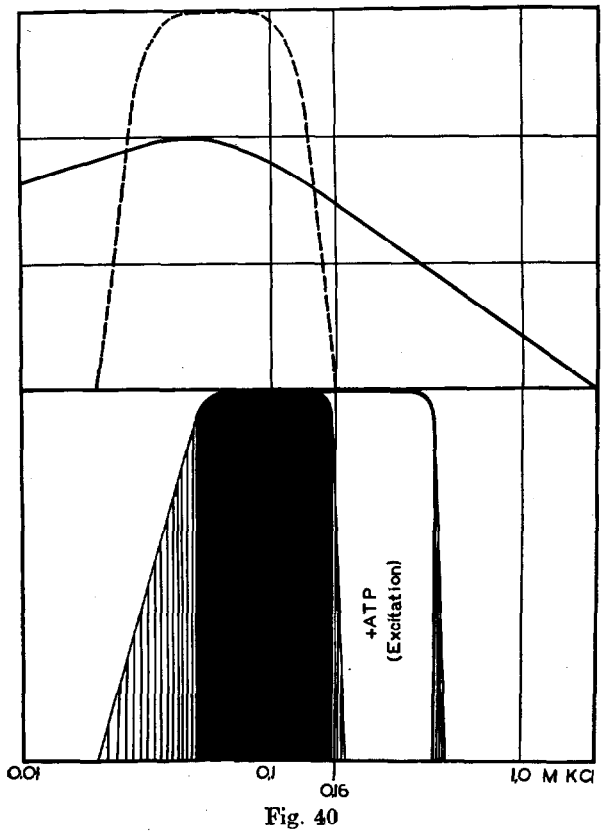

Fig. 40

If we suppose that ATP is responsible for the difference, then we must assume it to be in some inactive state in resting muscle. Knowing that the ATP present in muscle is adsorbed to myosin, we may formulate the situation by saying the adsorbed ATP causes contraction only below 0.16 M KCl, while free, added ATP does so up to 0.45 M. Hypotonicity activates the ATP by releasing it from its adsorption. It was shown

before that the adsorption of ATP is dependent on ionic concentration.

Why adsorbed ATP is unable to cause contraction is but a matter of speculation at this time.

The simplest explanation is to suppose that ATP is adsorbed in a specific steric orientation, in which its active group is kept out of touch with the protein, pointing away from it.

It is simple to convince oneself of the effect of steric orientation on biological activity, by sitting on drawingpins.* The effect will be different if the pins are oriented by means of their flat heads (adenyl group) or randomly distributed with some points (pyrophosphate group) towards our surface.

III. Excitation

Buchthal, Deutsch and Knappeis (1944) discovered that ATP, applied to the isolated muscle fibre or given intra-arterially, produces twitches or a tetanus-like contraction which, in many ways, resemble normal contraction. They arrived at the conclusion that ATP is involved in the process of excitation and that the ATP, present in resting muscle, is present in an inactive condition, conclusions supported by our observations. Buchthal and his collaborators applied the ATP from a micropipette to certain points of the muscle fibre; the quantity of ATP was small but its concentration high.

Unaware of these experiments, G. Rózsa started work on similar lines with a somewhat different technique. He suspended the isolated muscle fibre in Ringer's solution, or slightly hypertonic (0.2–0.25 M) KCl, and added ATP. So in these experiments ATP acted in a uniform concentration on the whole fibre and produced permanent shortening. Rózsa used freshly isolated muscle fibres and found that 0.5 γ of ATP per ml. sufficed to cause contraction. This result was very remarkable for several reasons. It showed that ATP, in resting muscle, must be adsorbed completely; the concentration of free ATP cannot exceed 0.5 γ per ml., or else it would cause contraction. But this result was in contradiction to our previous experience which showed that actomyosin needs a high ATP concentration

*Thumb tacks.

for contraction; actomyosin will not contract if the ATP concentration sinks below 0.01% which is still more than one hundred times 0.5 γ per ml. This shows that these small concentrations of ATP added did not elicit contraction directly, but activated the relatively large quantity of ATP present in inactive condition, so that the ATP present in the fibre now behaved as free ATP, added from without, causing contraction up to 0.45 M KCl (Fig. 40).

The results were different if muscle fibres were used which have lost excitability. In these experiments the fibres were isolated after the muscle had lost electric excitability on storage. If such unexcitable fibres were used, ATP still produced contraction, but only in the high concentration usually needed to make actomyosin contract (0.01–0.1%). This suggests that the small amounts of ATP added to excitable muscle caused contraction by acting on the normal mechanism of excitation which activates the ATP present in resting muscle.

Whatever the mechanism may be, the observations presented make it sufficiently clear that the relaxed actomyosin micel, in muscle, has a very complex and labile structure, extremely delicately balanced. The stable, energy-poor condition is the contracted one. The muscle fibre contains 5,000 times more ATP than is necessary to elicit contraction. The adsorption (inactivation) of this ATP depends on K adsorption, which property is very labile. The effect of K adsorption, in its turn, depends on the previous adsorption of bivalent ions. Under certain conditions, the autocatalytic release of ATP may be started by the release of one single K ion and the loss of the bivalent ions may lead to the release of all the adsorbed ATP, K being inactive without bivalent ions. Sufficient evidence was given that these ions may influence each other's activities in a subtle way. An equally subtle property of actomyosin is its dissociation which depends both on ions and ATP. Moreover it has been shown that isotonicity means a critical ionic concentration and the whole equilibrium collapses on slight dilution. Thus, though we are unable to say what happens on addition of 0.5 γ ATP per ml., we know that resting muscle represents a most subtly balanced metastable condition and we cannot

be surprised if a small quantity of ATP is capable of upsetting it; or if the equilibrium is disturbed by an electric shock, which might tear off one K^+ or distort electric fields. Nor can we be surprised if the changes occurring in one micel upset conditions in its neighbor. Possibly the ATP released in one micel may also start the process in its neighbor, or it may be that the water released by one contracting micel causes dilution and herewith contraction of its neighbor.

There is one more point which should be mentioned. The observations suggest that the autocatalytic release of ATP plays a role in the normal process of excitation. It has been mentioned that a minute quantity of ATP, added from without, will cause contraction in the excitable muscle fibre even if 0.25 M KCl is used as suspension fluid. This is remarkable because muscle readily loses excitability in KCl. It may be deduced herefrom that KCl inactivates that part of the normal mechanism which liberates the first quantity of ATP but does not interfere with further action of this ATP. Possibly this KCl prevents the release of ATP by making its adsorption too strong.

Mention should be made, finally, of experiments of Rózsa, who has also studied the action of a series of poisons known to cause contraction in muscle. The freshly isolated muscle fibres were suspended in 0.25 M KCl and the poisons were added. Acetylcholine and nicotine caused no contraction. Eserine was active in $1:10^7$, veratrine in $1:10^6$, quinine and caffeine in $1:10^4$ dilution. What gave special interest to these experiments is the fact that these poisons were active only in excitable muscle. As soon as excitability was lost the poisons became inactive. These drugs seem thus to act by setting into action the normal mechanism of excitation, activating, in some way, the ATP present.

IV. Contraction

If the muscle fibre is subjected to a rather rude treatment, designed to destroy all finer structure, retaining only the rigid actomyosin system, and the fibre thus treated is suspended in dilute KCl and ATP is added, it contracts violently. So, for

instance, if the muscle is washed in distilled water for several days at 0°, then frozen on the freezing-microtome, cut into slices parallel to the fibres, thawed and suspended in KCl, it contracts violently if ATP is added, similarly to threads of actomyosin. This reaction is very specific and no other substance will elicit such contraction except ATP, the normal constituent muscle, or substances closely related to it. Evidence indicates that the two phenomena, the contraction of actomyosin threads and the contraction of muscle fibres in ATP, are related.

Contraction of actomyosin seems to be a simple colloid-chemical process, synaeresis, taking place in a specific structure, built of two specific colloids. In muscle the steric orientation, called "structure," may still add to the specificity of the reaction, but there can be little doubt that in essence both contraction of muscle and contraction of actomyosin are identical phenomena. By orienting the particles within threads the similarity of both processes can still be increased.

In the first part of this book a theory was evolved which attributes the special features of the contraction of actomyosin to the bending of particles, or angular motion. This theory, if applied to muscle, explains different features of muscular contraction in a rather satisfactory manner. It explains why muscle becomes shorter and thicker, and why it loses DR on contraction. It answers an old puzzle. In colloids, long-range forces are very weak and may lead to the accumulation of much intermicellar water. The loss of this water may give very extensive shrinking but is inadequate to do work. Short-range forces may be made to do work but the corresponding changes in volume are small. Muscle performs very extensive monodimensional shrinking but does considerable work throughout. This is nicely explained by our theory according to which this this motion is but the magnified action of the short-range forces.

Another puzzle is: why is the muscle strongest in its most stretched condition? If shortening is due to mutual attraction of atomic groups or particles, then why do these forces increase if we pull these groups or particles apart? It is in love only that attraction increases with the distance. But if muscular con-

traction is an angular motion, then, for a given shortening, the motion of any point of the rod (Figs. 23–24) will be most extensive and the product, force × distance, will be the greatest in the straight condition.

All this agreement is very gratifying but one would like to have more direct evidence about the basic correctness of the theory. Unfortunately the Röntgen method, in its present form, seems to be inadequate for the study of contraction. As far as I can judge, its results are in harmony with our theory but the evidence is negative. At low degrees of contraction, 10–20%, we can expect only part of the fibres to be contracted and the X-ray picture to be unchanged. At higher degrees of contraction we can expect the picture to become blurred. This is actually what has been found (Astbury, 1946). According to our theory, at the maximum of contraction, particles must be oriented at 90° to their original position at rest (Figs. 26 and 29). This degree of contraction throughout the muscle cannot be achieved by physiological stimulation. It may be achieved by heat, and Astbury actually found myosin in this position in heat-treated muscle. In histological slices of contracted muscle, at points of maximum contraction, DR can be found to have assumed an opposite sign.

It is difficult to arrive at final conclusions without being informed about the simplest basic questions, like the dimensions of the myosin and actin particle, as well as their orientations. Astbury finds the polypeptide chain parallel to the fibre axis, but Kratky, Secora, and Weber's* measurements suggest that the myosin particle is elongated and is formed by the collateral association of polypeptide chains, the long axis of the particle being perpendicular to these chains. If this would be so, then the myosin particle could stand radially, while the polypeptide chain stands coaxially. The angular theory of contraction makes no predictions on this line.

According to Varga's measurements, contraction is an endothermic process. This seems to be in contradiction to A. V. Hill, who has shown that contraction is exothermic. The contradiction is only apparent. What Varga measured was the

*Personal communication from Professor H. H. Weber.

heat change of the elementary primary process, the internal rearrangement of the particle. This is followed in a secondary way by dehydration, contraction, and also by the enzymatic splitting of ATP. What Hill measured is the heat change of the whole process in the whole muscle. If any prediction could be made from Varga's result, it would be that the function of the electric organ of fishes involves a cooling down, the electric organ being a sort of muscle without contraction.

V. Relaxation

As shown before, contraction and relaxation are two distinct states of actomyosin and there is no in-between state: it is either Jack or Jill. It has also been shown that the contracted state is the energy-poor state and at 37° the free energy of the particle decreases by 7,000 cal. on contraction. It follows that we have to impart 7,000 calories' worth of free energy to the system if we want it to relax. It would be simplest to picture relaxation as the reversal of contraction, starting as soon as the necessary quantity of energy has been invested. This picture, however, would be incorrect. It has been shown that only F-actomyosin is contractile and that the F-actin is broken up in contraction into globules. The properties of G-actin and G-actomyosin are very different from those of the F-form. We have to distinguish between two forms of G-actomyosin: the hydrated-relaxed, and the dehydrated-contracted form. (The latter can be obtained by making F-actomyosin contract.) The properties and reactions of these two forms are different. At *a medium salt concentration* the contracted-dehydrated, energy-poor, G-actomyosin is not dissociated by ATP. The relaxed-hydrated, energy-rich form is dissociated by ATP at any salt concentration. What we can expect to happen is the following: F-actomyosin is brought to contraction, going over into the energy-poor, dehydrated form. At the same time, the actin breaks up into globules. By going over into the contracted state the actomyosin becomes enzymatically active, splits ATP, going back into the energy-rich hydrated form. Under the action of ATP, this actomyosin dissociates into free G-actin

and free myosin. The G-actin then polymerizes into F-actin; we return to our starting point and the system is ready for a new contraction. This dissociation of the compound may greatly facilitate relaxation mechanically by decreasing the internal friction of the system which, in the contracted state, must be very high. But the dissociation may also facilitate return to rest chemically. It has been shown that free myosin has a greater adsorption power than has actomyosin. If the primary change in contraction is liberation of adsorbed ions or ATP, the readsorption and return to rest must be greatly facilitated by dissociation.

VI. Rigor and Contracture

In muscle every UW of myosin has one molecule of ATP adsorbed which contributes to the charge and hydration of the protein and herewith to the suppleness and plasticity of muscle. *Post mortem* this ATP is gradually decomposed and in the end we are left with a rather stiff, salt-precipitated actomyosin gel. Since dehydration means contraction, this relatively weak salt-precipitation will entail a weak contraction. This slightly contracted, rigid condition of actomyosin is *rigor mortis*. The actomyosin of such muscles is insoluble in 0.6 M KCl, no ATP being present; it can be rendered soluble again by the addition of ATP to the KCl solution [Erdös, 32].

Erdös has followed quantitatively the development of rigor and the disappearance of ATP. He has found that disappearance of ATP and stiffening of muscle set in immediately after death, progressing slowly until the complete exhaustion of ATP and the complete development of rigor. The two curves, that of the disappearance of ATP and that of the developing stiffness, were perfectly parallel. This shows that the whole physiological ATP saturation is needed to keep the muscle in a perfectly relaxed condition. The disappearance of the rigor, which occurs spontaneously later, was found to be due to the disintegration of the system, the actin becoming soluble. Exhaustion of ATP in an unexcitable muscle is rigor.

The picture will be different if ATP is exhausted *in vivo* in an excitable muscle. As shown before, less ATP is needed to produce contraction of actomyosin than is necessary to produce relaxation, and reduction of ATP concentration favors contraction at the expense of relaxation. If the concentration of ATP is decreased during excitation, the muscle will become more and more unable to relax and will stop at last in contraction. This condition also develops gradually. Erdös has compared the development of contraction and disappearance of ATP in different forms of contraction, like that developed under the action of monoiodo-acetate, chloroform vapor, caffeine, and prolonged labor. In all cases there was a parallel between contraction and ATP concentration. Though this parallel was less perfect than in the case of rigor, it was definite enough to show the close relation of the two processes. Exhaustion of ATP in excitable muscle is contraction.

The observations warranted the question whether certain pathological conditions, characterized by the failure of certain muscular elements to relax, are not due to the lack of ATP. Such conditions are, for instance, angina pectoris, certain vasospastic gangrenes, and dysmenorrheas. It was found at the medical clinic of Szeged that these conditions could be relieved by the administration of ATP. The experience showed that during treatment there were no attacks of angina; gangrenes which would have otherwise required amputation were healed; and dysmenorrheic pains were relieved. More extensive experience along these lines is desirable.

VII. Contraction, Fermentation, and Oxidation

The two sources of animal energy are fermentation and oxidation. The energy of fermentation is made available to the contracting mechanism in the form of the phosphate bonds of ATP. If the muscle needs energy it splits these bonds and the dephosphorylated ATP sets the mechanism of fermentation going by serving as phosphate-acceptor. Contraction sets off dephosphorylation and dephosphorylation starts fermentation.

The need for energy is the automatic starting mechanism for both processes.

In fermentation, the bound phosphate is not transported to myosin by the ATP. As shown, resting muscle can contain no free ATP: the ATP must be adsorbed. Most of it is linked to myosin; the rest, to the enzymes of fermentation. The ATP is released by myosin only after dephosphorylation and deamination to inosinic acid which has no more biological activity. The role of phosphate carrier is fulfilled by creatine. The phosphate is transferred from ATP to creatine and from creatine to ATP by a special enzyme, the function of which was described by Lohmann in the following equation:

$$\text{ATP} + 2 \text{ creatine} = \text{AMP} + 2 \text{ creatine-phosphate.}$$

It has been shown by Banga [34] that the Lohmann reaction is actually performed by two enzymes, one of which establishes equilibrium between ATP and creatine:

$$\text{ATP} + \text{creatine} \rightleftharpoons \text{ADP} + \text{creatine-phosphate}$$

and one which establishes equilibrium between ADP and creatine phosphate

$$\text{ADP} + \text{creatine} \rightleftharpoons \text{AMP} + \text{creatine-phosphate.}$$

She called the first ATP-creatine phosphopherase, the second ADP-creatine phosphopherase. The first was separated from the second and its equilibrium-constant established (Table V).

TABLE V. *Experimental Technique: 1 ml. of Veronal Acetate Buffer Solution of pH 8.55 + 0.1 ml = 60 γ ATP-phosphopherase + Varying Quantities of ATP and of Creatine*

M creatine added	M ATP added	M P found	$K = \frac{\text{Cr.P.ADP}}{\text{ATP.Cr.}}$
0.02390	0.00222	0.00104	0.040
0.02390	0.00444	0.00158	0.042
0.02390	0.00890	0.00232	0.038
0.02390	0.01331	0.00292	0.039
0.01030	0.00236	0.00078	0.040
0.01531	0.00236	0.00090	0.038
0.02040	0.00236	0.00104	0.040
0.02542	0.00236	0.00108	0.038

Volume: 1.6 ml. Incubation at 38°, until state of equilibrium had been reached.

This constant varied with the pH and reached maximum at the pH-optimum (pH 9)of the reaction. These enzymes are very powerful and readily transfer the phosphate of ATP to creatine at the sites of its production (fermentation) and from creatine-phosphate to ADP at the sites of its utilization (contraction).

The relation of oxidation and contraction is less clearly understood. Oxidation could not be the sole source of the energy of contraction because the function of muscle is so abrupt that oxidation could not always keep up with it, and there has to be a readily accessible store of reserve energy, independent of 0_2 supply. This store is the bound phosphate of ATP and phosphagen (phosphocreatine). This, of course, is no reason for the muscle not to use oxidative energy too.

Other, more steadily working organs, like the kidney or brain, seem to depend entirely on oxidation for their energy. We can thus expect that muscle too will do the same and use phosphate if oxygen supply becomes inadequate. It becomes increasingly probable that muscle even uses its oxidative energy to synthesize bound phosphate, as seems to be the case in liver and kidney (Kalckar). It is even possible that there is a fluid equilibrium between phosphate splitting, phosphate synthesis, oxidation, and the energy state of actomyosin. If actomyosin is in its low-energy state of contraction, it splits phosphate; if it is in its high-energy state, it synthesizes it, taking the excess energy from oxidation. It may be brought from the low, contracted level to the higher, relaxed level either by phosphate splitting or oxidation.

These ideas are somewhat ahead of the experimental evidence, and coming back to earth, our first question may be: is there any close relation between the contractile system, its function, and oxidation?

The furnace in which the H of the foodstuff in muscle is burned is composed of the oxidases of succinic and citric acids. Both the succino- and citrico-dehydrogenase are linked to the water-insoluble structure and we have to suppose that either the oxidases are linked to the actin-myosin system, or else at the side of actomyosin there is another structural system to bear them.

Biró and A. E. Szent-Györgyi have undertaken to decide between these two possibilities. Knowing that the physical state of actomyosin is governed by ions and ATP in a rather specific way, they asked how far in washed muscle the oxidation of succinic acid depends on the presence of ions and ATP and whether there is any relation between oxidation and the physical state of actomyosin.

Actomyosin is precipitated (contracted, dehydrated) to some extent by KCl with a maximum of about 0.05 M. ATP very greatly increases this reaction up to 0.5 M KCl, where the system disintegrates. The results of Biró and Szent-Györgyi are summed up in Fig. 41. There is a complete parallelism between succinoxidation and the physical state of actomyosin. As the curve "K" shows, the oxidation of succinic acid depends on the K concentration and has a maximum about 0.05 M KCl where actomyosin is isoelectric and is maximally precipitated. The enzymatic activity is very greatly enhanced by ATP (curve ATP). Ca and Mg make the curve of enzymatic activity steeper, the zone narrower, as they do in the case of contraction. At the higher KCl concentrations, oxidation stops where the

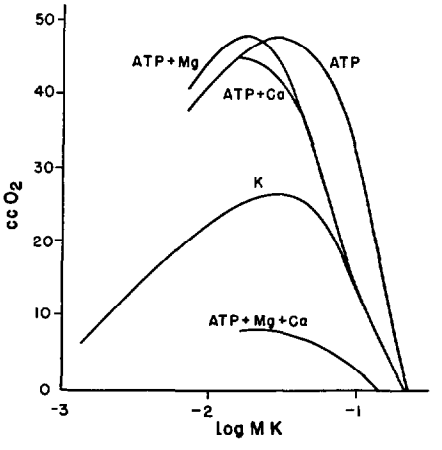

Fig. 41. Oxygen uptake of 300 mg. of washed muscle at varied K concentrations in absence and presence of 0.125% ATP, 0.005 M $MgCl_2$, 0.005 M $CaCl_2$, 1.2 mg. succinate. Abscissa: log M K present. Ordinate: cc. O_2 adsorbed.

actomyosin system disintegrates. The curve rapidly falls to the left of the maximum towards the smaller K concentration. (In the presence of ATP these curves could not be extended any further since K had to be introduced as cations of the ATP.)

The analogy of oxidation and the functional state of actomyosin is drawn still closer by the simultaneous action of Ca and Mg. Both ions have a strong promoting effect on the phosphatase activity of actomyosin, but have a very strong inhibitory effect if present simultaneously. This reaction is very specific and the curve Mg+Ca shows that oxidation of succinic acid is also very strongly inhibited by the combination of both ions.

As far as experience can take us, there is thus a complete analogy between the state of actomyosin and oxidase activity. Where there is partial contraction (KCl alone) there is partial activity; where there is maximal contraction, there is maximal activity; and where there is no contraction, there is no activity. In the presence of ATP there can be no partial contraction, only complete relaxation or complete contraction, as far as single micels are concerned; there can thus be no partial activity either, and only the contracted micels are oxidatively active.

These results show that actomyosin is involved in succinic oxidation. The oxidase and the contractile matter form one single functional system together. This must seem rather natural to the physiologist: what logic would there be in having the energy liberated by another system than that which uses it?

Our second conclusion, that the system is enzymatically active only in its contracted state, may seem still stranger to the enzymologist, but to the physiologist it will seem natural that the system should liberate energy only when needed, when in its lower energy-level. This is again an expression of the same fundamental principle we have met in phosphate hydrolysis: it is the need for energy which regulates its liberation. The liberation of energy starts automatically if energy is needed, and stops automatically as soon as energy becomes superfluous.

One wonders about the inner mechanism of this regulation. Biró and Szent-Györgyi have tried to approach this problem

by finding out which part of the succinoxidase system is responsible for it. The succinoxidase system is composed of three parts: at the one end there is the oxygen activation (*Atmungsferment*, cytochrome oxidase). At the other end there is the hydrogen activation (succino-dehydrogenase). In the middle there is the series of cytochromes which transmits the electrons, liberated by the dehydrogenase, to oxygen, activated by the cytochrome oxidase. The experiments showed that neither oxygen activation nor hydrogen activation are dependent on ATP or concentration of KCl. Naturally, this does not mean that there is no close relation between these enzymes and actomyosin; the experiment simply says that the enzymatic activity does not depend on the functional state of this protein.

Biró and Szent-Györgyi performed a great number of experiments to convince themselves either of the identity or the independence of the dehydrogenase and actomyosin. They followed two methods. They prepared "dehydrogenase" from heart by the standard method: the final preparation had all the properties of actomyosin. On the other hand, they tried to separate dehydrogenase activity from actomyosin and to prepare dehydrogenase-free myosin, or myosin-free dehydrogenase, but were unsuccessful. Their results, at this point, were rather complex and were largely in agreement with the assumption that dehydrogenase activity is linked to actomyosin which is the most active when present in its original structure, as it is in muscle. Once this structure is disrupted, myosin carries with it the activity, which becomes very labile. The activity cannot be increased by the addition of actin but is higher the more actin the myosin originally contains.

Having shown that it is neither the oxygen nor the hydrogen activation which depends on contraction, we are led to conclude that the electron-transport system is broken up in relaxation and restored in contraction. This means that there is always active oxygen and active hydrogen, but in resting muscle they do not react since the bridge between them is broken. As to the mechanism of the inactivation and activation of the electron transport, we can only guess. It may be that in the relaxed state the electronic structure of the system is simply such that it

will not work. But there is also a simpler and cruder mechanism possible, and we may think that by the stretching out and hydration of the myosin particle the cytochromes lose touch and become active again if the system goes back into its dehydrated-contracted state which brings the cytochromes into touch again.

Two objections may be raised: how is it possible that the action of metal ions has hitherto been overlooked? Succinoxidase is one of the most classical objects of research and many workers used distilled water as suspension fluid. The probable explanation is rather simple: succinate was added in these experiments as a K or Na salt and herewith the necessary metal was introduced, usually in optimal concentration.

The other objection may be this: the oxidative resynthesis of lactic acid takes place in the recovery period, when actomyosin is relaxed. It is possible that at the side of succino- and citrico-dehydrogenase there is another oxidative system (diaphorase?) involved in lactic oxidation which is independent of actomyosin or its functional state.

VIII. Lipins, Smooth Muscle, Heart Muscle, Kidney, and Brain

Myosin, recrystallized repeatedly, still contains a considerable quantity (3%) of lipid matter, partly soluble, partly insoluble in acetone. Lipids seem to play an important role in the structure of the contractile system, being closely associated with myosin. The acetone-insoluble fraction contains two main substances, one a cerebroside, which has been obtained in crystalline condition, and the other a substance which seems to be an alcohol-soluble cephalin. The latter, as shown by B. Jánszky (oral communication) is very active as thrombokinase while the former seems to be involved in the different functions of myosin. It is fairly difficult to liberate myosin from the cerebroside but, as shown by F. Guba, this can be achieved by washing the myosin with rather large volumes of fluid. Such a myosin will not contract with ADP but does so after the addition of the cerebroside, provided that protein II is present. A prelimi-

nary experiment of Biró and A. E. Szent-Györgyi indicated that its presence is necessary for dehydration of succinate also. All these are but suggestions, showing that the study of lipins is a rather large and fascinating field. Cortical hormones of the adrenal gland, which have such a profound influence on the development and function of muscle, will also have to be fitted into the picture.

Another most fascinating problem is to find out how far the relationships found in muscle represent general principles of living matter, or how far they are specific cases only. Some observations suggest that the different functions of different organs are closely related, with the same basic mechanism adapted to specific purposes. Caffeine, which produces contraction in muscle, produces increased nervous and renal activity. Veratrine, which provokes protracted contraction in muscle, produces prolonged flow of saliva. If the same key opens different slots, the mechanism of these slots cannot be any too different. The same holds true for the action of ions. It is the same ionic balance in different organs which conditions normal activity.

When trying to apply our experience gained on cross-striated muscle to other organs, the logical sequence is to start with the most closely related tissue, i.e., heart and smooth muscle. Rózsa [51] subjected these to careful study. He prepared actin and myosin from them, and studied the properties and reactions of the separate components as well as the actomyosin combination. He also tried to combine smooth-muscle myosin with cross-striated-muscle actin and *vice versa*. He studied the contractility and reactions with ions and ATP. His results can be summed up by saying that he found no real difference between the three sorts of muscle, cross-striated, heart, and smooth muscle. The difference in the function of these three different organs is thus not a difference of this basic contractile mechanism, but a difference in higher organization and regulation.

There are differences in the extractibility of myosin and actin in different varieties of muscles but, as shown by Guba [70] there are considerable differences in this respect between the cross-striated muscles of different animals or even between the different body muscles of the same animal.

Lajta [59] undertook to study a quite different organ, the kidney. On first approach there is a great similarity between the behavior of kidney and muscle. If the freshly minced muscle is suspended in a strong salt solution (0.6 M KCl) a viscous extract is obtained (containing myosin and actomyosin). The dissolution of the viscous structural protein is due not merely to an action of the salt but also to the action of the bound phosphate of the ATP present. If the minced muscle is stored, the ATP decomposes and the protein becomes insoluble. Instead of a viscous extract, a thin liquid of low viscosity is obtained. Parallel to this change, the bound phosphate disappears and is found now in free condition.

If freshly minced kidney is suspended in 0.6 M KCl, a very viscous and thixotropic extract is obtained. If the minced kidney is stored before extraction for an hour at 37° or for twelve hours at 0°, the extract will not show great viscosity. At the same time the free phosphate increases at the expense of bound phosphate to about the same extent as in muscle. The difference between fresh and stored kidney is still more marked if, instead of 0.6 M KCl, a 2 M KCl or alkaline KCl containing 30% urea is employed.

Rabbit muscle contains, on the average, 2.5 mg. ATP per g. with about 0.25 mg. labile phosphate, which is liberated on storage. In kidney, during storage for one hour at 37°, an average of 0.3 mg. P was liberated per g. of tissue. This phosphate, however, is not derived from ATP (there can be but very little ATP in kidney!) but from the protein and is linked in the fresh tissue to the structure itself, being found in the trichloracetic-acid-insoluble fraction.

These results indicate that, as in muscle, the physical state of the structural protein of kidney is decided by the bound phosphate, but contrary to muscle this bound phosphate is not linked to a small molecule adsorbed to protein, but to the protein itself.

The K ion plays a basic role in muscle, and actomyosin has a very high adsorption power for this metal. Is high K-adsorption power connected with the specific function of actomyosin or is it a general property of structural proteins, connected

with the nature of life itself rather than with a specific function only? In order to approach this question, Lajta measured the K-binding capacity of kidney and brain. He washed the freshly minced organs with 0° water, suspended the mince

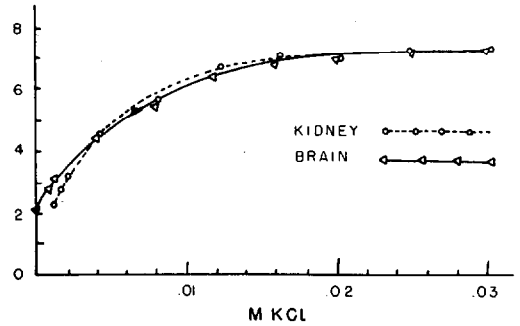

Fig. 42. K adsorption curve of washed kidney and brain. Ordinate: equivalents of K bound per UW of water-insoluble structural protein.

in KCl solution of different concentrations, and precipitated the suspension with alcohol, as done by Banga and Hermann in estimating the K fixation by myosin or muscle. Above 0.3 M the precipitate contained Cl, so the K fixation was followed only up to this concentration. His results are summed up in Figures 42 and 43. The curve, like that of myosin, corresponds

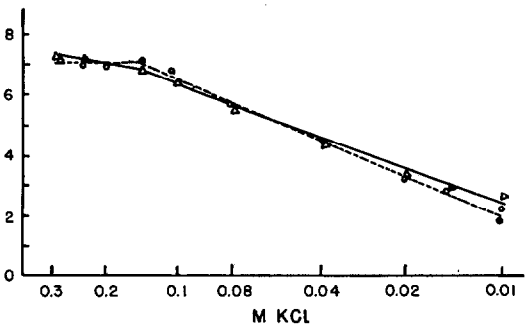

Fig. 43. Same as Fig. 42 on logarithmic scale.

to an exponential equation, being straight on logarithmic scale. It shows a break at the isotonic concentration. Here too the break probably occurs at the IP, but unlike myosin there is no more adsorption above this point. The break is

reached with seven equivalents of metal adsorbed. If fresh kidney or brain is precipitated with alcohol, three K ions in kidney and four in brain are carried down with the protein. If the K adsorption, in washed tissue, is effected in the presence of 0.001 M Mg, one K less will be bound.

The results show that similarly to muscle, the structural proteins of kidney and brain also have a great affinity for K and are capable of binding this metal, and that here too the isotonic, ionic concentration marks a turning point, though the rules governing this adsorption are somewhat different than in muscle, according to the different special functions of these organs.

IX. On the Nature of Cross-Striation

There are two trends at present in explaining the cross-striation of muscle. The morphologist's view is that muscle is what it looks like: a structure built of segments, comparable to a roll of different coins put together in regular sequence. If this is correct, cross-striated muscle represents a very highly differentiated structure. It is difficult to see how such a highly differentiated structure could appear so suddenly, without any transitory forms, in the phylogenetic scale and how and why heart muscle fibres, in tissue cultures, should first begin contracting and later develop cross-striation. The anatomist's view has found the support of the biochemists who showed that the different segments have different chemical composition. These differences, however, were detected by means of optical methods — microscope, spectroscope, or polarimeter — and might have thus been subjected to the same error as direct visual observation. They cannot be adduced as independent arguments.

The other view is that cross-striation is but an optical phenomenon due to some sort of periodically changing property of a continuous structure. Bernal explained cross-striation tentatively, ascribing to fibrils the structure of reversed spirals. I myself have tried to explain cross-striation by a spiral

structure of elementary fibrils [70]. Neither of these theories can be maintained in the light of recent observations with the electron microscope (Hall, Jakus, and Schmitt) and there is no use in speculating about the nature of cross-striation until the possibilities offered by this splendid, new method are exhausted, and we are better informed about the dimensions of the actin and myosin particle.

I myself tried to decide between the spiral and segmental theory by rotating the insect muscle-fibril under the microscope: if the cross-striation is due to segmental structure, rotation should make no difference; if it is due to the spiral structure, striation should move on rotation along the axis. I have seen it moving, and moving just the distance demanded by the theory (two segments on a full rotation). These experiments, however, were done under very bad conditions which did not allow repetition or spotless objective registration; and knowing the difficulties of the experiment I must say myself that the result cannot be accepted as final until corroborated. I published the results, not expecting to live to repeat them [70]. I found the striation in half of the fibres moving from left to right, in the other half from right to left, which indicates partly left, partly right spirals.

At the moment the whole problem is in flux. The electron microscope pictures of Hall, Jakus, and Schmitt indicate that the fibril is built of a great number of still smaller filaments of 50–250 Å diameter, similar to the threads, observed by Ardenne and Weber as well as by the American workers on "myosin." The pictures suggest that cross-striation is rather a property of the substance located between filaments.

I was often amazed to find under the microscope cross-striation and fibrillary structure still present, apparently unaltered, in muscle fibres from which the bulk of myosin has been extracted.

It is possible that cross-striation is the result rather than the cause of motion, like the cross-striation in van Iterson's streaming colloids and Bernal and Fankuchen's vibrating virus solutions. That something is wrong with the old morphologist's view is clearly borne out by the simple observation

that I-bands do not contract, but rather expand in contraction. Since they make half of the length, muscle could never contract by more than 50% if the I-bands were anatomical structures. Muscle may contract to one-fifth.

This much may suffice to bring out the interest of the prob-

TABLE VI

Name of Insect	Musculus adductor mandibulae	Musculus extensor tibiae	Musculus dorso-ventralis	Remarks
1. *Decticus verrucivorus* ♂	5.4	2.7	2.0–2.7	wings unfit for flying; jumper legs
2. *Locusta viridissima* ♀	5.4	2.7	2.7	larva, unable to fly, jumper legs
3. *Stenobothrus sp.*	—	1.4–2.7	—	larva, jumper legs
4. *Liogryllus campestris*	5.4	4.0–5.4	2.7	jumper legs
5. *Carabus Scheidleri jucundus*	2.7–4.0	5.4	5.4	no wings
Carabus Scheidleri jucundus	5.4–8.1	5.4	4.0	no wings
6. *Cybister laterimarginalis*	5.4–6.7	2.7–4.0	2.0–2.7	swimmer legs
Cybister laterimarginalis	4.0–6.7	2.7–4.0	2.7	swimmer legs
Cybister laterimarginalis	5.4–8.1	4.0	2.0–2.7	swimmer legs
7. *Hydrous piceus*	8.1–10.8	5.4	4.0	
Hydrous piceus	5.4–8.1	5.4–6.7	1.3–2.7	
8. *Gnaptor spinimanus*	5.4	5.4	4.0–5.4	no wings
9. *Anoxia orientalis*	8.1	3.5–5.4	—	
10. *Lucanus cervus* ♂	6.8–8.1	4.0–5.4	1.7	big
Lucanus cervus ♂	8.1	5.4	2.0–2.7	medium size
Lucanus cervus ♂	8.1	5.4	1.3–1.7	small (ab. capreolus)
Lucanus cervus ♀	8.1–10.8	5.4	1.0–1.5	
11. *Cerambyx cerdo* ♂	5.4	4.0	1.3–2.7	
Cerambyx cerdo ♂	5.4	5.4	1.3	
12. *Sphinx convolvuli*	/5.4/*	2.7–4.5	1.3–1.7	*Musculus dilatator pharyngis
13. *Apis mellifica*	—	2.7–4.0	2.0–2.7	
Apis mellifica	—	2.7–4.0	2.7–4.0	
14. *Polistes gallica*	5.4–6.7	2.7–4.0	2.7–4.0	
15. *Bombus lapidarius*	—	4.0–4.5	2.0	
16. *Aedes vexans*	—	1.7	1.7	
Aedes vexans	—	1.5–2.0	1.7	

1–4 Orthoptera, 5–11 Coleoptera, 12 Lepidoptera, 13–15 Hymenoptera, 16 Diptera. Numbers indicate the diameter of one Q and I band in 0.001 mm.

lem as well as our ignorance. More definite data are urgently needed.

I will close this part of my book with the very fascinating data of W. Szekessy [52] who asked himself whether there is any relation between width of cross-striation and the rate of motion. There is a very wide range of variation in the rate of motion of different insect muscles. The mandibular muscles, moving the jaw, move exceedingly slowly. The legs are much faster, and the wings are the fastest. Székessy's measurements, summed up in Table VI, show that the widest striae are found in mandibular muscles, where the diameter of one period may exceed 10 μ; the average diameter was found to be 6 μ. The diameter in leg muscle averages 4 μ and that of thoracic muscles, moving the wings, is 2 μ, on the average. Thoracic muscles in animals which do not fly (*Carabus, Gnaptor*) have considerably wider striation.